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Helminth parasites – masters of regulation

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Summary: Immune regulation by parasites is a global concept that includes suppression, diversion, and conversion of the host immune response to the benefit of the pathogen. While many microparasites escape immune attack by antigenic variation or sequestration in specialized niches, helminths appear to thrive in exposed extracellular locations, such as the lymphatics, bloodstream, or gastrointestinal tract. We review here the multiple layers of immunoregulation that have now been discovered in helminth infection and discuss both the cellular and the molecular interactions involved. Key events among the host cell population are dominance of the T-helper 2 cell (Th2) phenotype and the selective loss of effector activity, against a background of regulatory T cells, alternatively activated macrophages, and Th2-inducing dendritic cells. Increasingly, there is evidence of important effects on other innate cell types, particularly mast cells and eosinophils. The sum effect of these changes to host reactivity is to create an anti-inflammatory environment, which is most favorable to parasite survival. We hypothesize therefore that parasites have evolved specific molecular strategies to induce this conducive landscape, and we review the foremost candidate immunomodulators released by helminths, including cytokine homologs, protease inhibitors, and an intriguing set of novel products implicated in immune suppression.

Introduction

Parasitic helminths represent an extreme in the spectrum of pathogens, as large multicellular animals derived from free-living metazoan ancestors. Although commonly grouped together, the helminths in fact comprise two very distantly related taxa that diverged 600 million or more years ago (1): the roundworm nematodes and the flatworm platyhelminths. Between these two main groups of distantly related helminth parasites, individual species of parasites have evolved to occupy a diverse range of niches within their hosts, using a wide range of infection strategies, yet with few exceptions the mammalian host responds to these diverse groups of organisms in a remarkably consistent and even stereotypic manner (2). Typically, this response involves the production of the cytokines interleukin-4 (IL-4), IL-5, IL-10, and IL-13, as well as immunoglobulin E (IgE) and the expansion and mobilization of specific effector cells, such as mast cells,

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eosinophils, and basophils. Collectively, this group of responses is known as the T-helper 2 (Th2) immune response (3).

A question that then arises is why do such distantly related parasites generate such similar host immune responses? It is possible that the immunological similarities which exist between these groups reflect shared helminth molecular ‘identity markers’ to the host immune response (4, 5), similar to the Toll-like receptor system by which microbial pathogens are recognized. However, it may also reflect parallel evolution of common strategies to exploit loopholes in the host immune response, either for the creation and maintenance of a beneficial environment within the host or for the suppression of host defense mechanisms. Indeed, the thesis of this review is that helminths of both types have discovered, and taken advantage of, the Achilles’ heel of the immune system – the self-imposed system of immune regulation that protects us from lethal autoimmunity.

The long life span of helminth parasites is evidence enough that they are accomplished at immune evasion, and it is clear that interference and modulation occur from the very first events in infection. Helminths do not simply ward off immune attack; rather, they influence and direct immune responses away from the modes most damaging to them, regulating the host immune response to create niches that optimize successful feeding and reproduction. Their means of accomplishing this feat are the subject of this review. Below, we discuss how helminths induce remarkably strong Th2 responses and the factors that restrain Th2 immunity from eliminating target parasites. We then discuss our most recent data demonstrating the crucial role of regulatory T cells in restraining host responses both to parasites and to third-party antigens such as allergens. We then distinguish between ‘effector’ Th2 activity and the downmodulated (‘modified’ or ‘conditioned’) Th2 phenotype which accompanies active infection. A similar distinction can be applied to the macrophage population, which enters a state of ‘alternative activation’.

In parallel to these exciting insights into the cellular interactions in infection, understanding of helminth immunomodulation is emerging at the molecular level. Numerous parasite-derived proteins, glycoconjugates, and small lipid moieties have been discovered with known or hypothesized roles in immune interference. We discuss these in the final section of the review.

The immunoregulatory scene in human infection

A key point, often overlooked, is that infections with parasitic helminths are quite commonly asymptomatic, and most hosts

are able to tolerate the presence of parasites normally considered as ‘pathogenic’ for considerable time without ill effects. Significantly, pathology is more closely associated with heightened immunological reactivity as for example in hepatic disease in schistosomiasis or acute lymphatic inflammation in filariasis (6). The fact that the immune system is capable of reacting vigorously to the parasites and yet generally does not is one indicator that downregulation of responsiveness is occurring in helminth infection. Perhaps both host and parasite benefit in their own ways from this process: but is immunoregulation the autonomous decision of the host immune system or the result of an elaborate survival strategy on the part of the parasite?

The epidemiological picture of immune regulation in helminth infection is one which shows remarkable similarities between different infections such as filariasis and schistosomiasis (6, 7). Typically, individuals with heavy infections have compromised antigen-specific T-cell responses in peripheral blood populations (8–11), most evident in a lack of *in vitro* proliferation and diminished IL-2 and interferon- γ (IFN- γ) responses to antigen challenge. We have some insight into the dynamics of immune depression from animal models, in which early responsiveness gives way to immunosuppression as the infection progresses to the patent phase (12, 13), as exemplified in Fig. 1A and B. In heavy infections, immune downregulation extends to polyclonal mitogen stimuli (Fig. 1C), a finding we return to in the context of impact of helminths on bystander responses.

Reactivity is not, however, altogether absent: T cells do produce IL-4 in response to antigen *in vitro*, but IL-5 is (like IFN- γ) suppressed (14). Suppression is dependent on the continuing existence of parasites; *in vivo* T-cell responses are restored following curative drug treatment (11, 15), with the recovery of proliferation (Fig. 1D) as well as the Th1 and Th2 effector cytokines IL-5 and IFN- γ . These key observations lead us to conclude that inflammatory cytokines of both Th1 and Th2 type are concomitantly suppressed. The pattern of immune suppression in human helminth infection does not map neatly across a Th1-versus-Th2 pendulum, leading workers in the field to consider the activity of regulatory populations (16–18).

A critical series of experiments established that filarial- and schistosome-specific T cells are present in infected patients and that their reactivity can be uncovered in the presence of antibodies to the regulatory cytokines IL-10 or transforming growth factor- β (TGF- β) (19–21). As discussed later in this review, these data can now be interpreted not only as evidence for regulatory T-cell activity in human helminth infection but also as a reflection on the unresponsive state of parasite-reactive T cells.

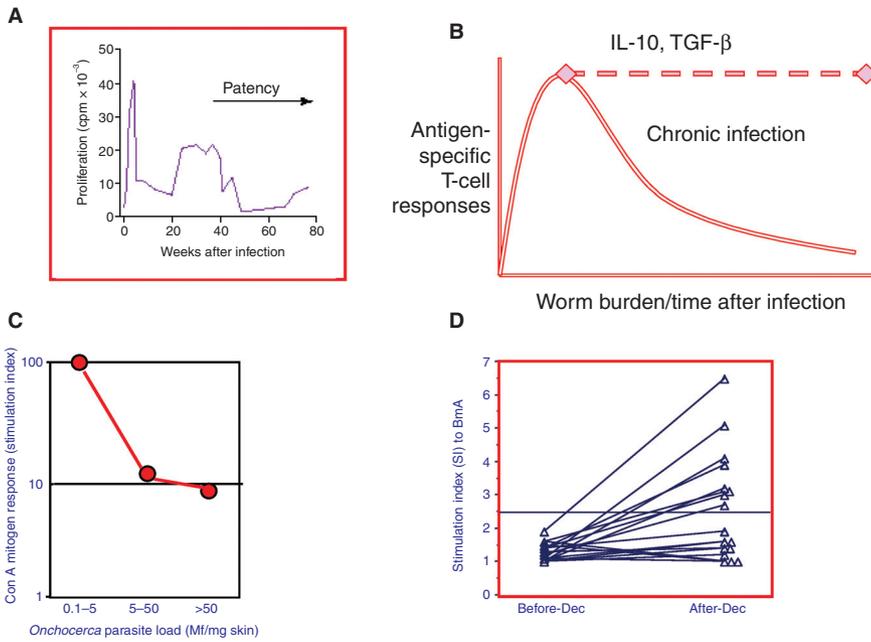


Fig. 1. Immune modulation in human helminth infection. (A) Time course antigen-specific proliferation in peripheral blood mononuclear cells from cattle infected with *Onchocerca* [reproduced from (13)]. (B) Schematic time course of immune suppression in filariasis, with reversal by neutralizing antibodies to interleukin-10 (IL-10) or transforming growth factor- β (TGF- β). (C) Spillover suppression of mitogen responses in onchocerciasis (data from M. E. Murdoch and R. M. Maizels, unpublished). (D) Reversal of unresponsiveness in *Brugia filariasis* following diethylcarbamazine (DEC) chemotherapy (16).

Learning from mouse models

A surprisingly wide choice of mouse model systems is available for helminth infections. Some, like *Schistosoma mansoni*, have been reviewed recently (22, 23), and within this review, we focus on our own work with nematode models of infection. A variety of parasite species (Fig. 2) display diverse migratory pathways and inhabit different niches (tissue compartments or gastrointestinal tract). In experimental models, some organisms achieve chronic infection while others are expelled, with the outcome often dependent on host genotype. Common to all systems, however, is some form of immune deviation or suppression.

Three species of gut nematodes act in different but instructive ways. *Heligmosomoides polygyrus* establishes long-term infections in most strains of mice, and it has a distinctly

immunosuppressive phenotype. *H. polygyrus* infection inhibits expulsion of other parasites, converting an otherwise short-lived *Nippostrongylus brasiliensis* infection into chronicity (24). At the cellular level, we have shown that regulatory T cells are stimulated in *H. polygyrus* infection (Wilson M., Finney C, & RMM, unpublished), while molecular studies have reported that parasites secrete a low molecular weight immunomodulatory factor that substantially inhibits *in vitro* lymphocyte responses (25). *N. brasiliensis* itself is short lived under standard high-dose infections, stimulating a powerful and protective Th2 response (26–29); however, low-dose (‘trickle’) infections more akin to natural rate of exposure develop into chronicity (30). A third system has been *Trichuris muris*, in which susceptibility is linked to the development of a Th1 response; mice that fail to mount a protective Th2 response

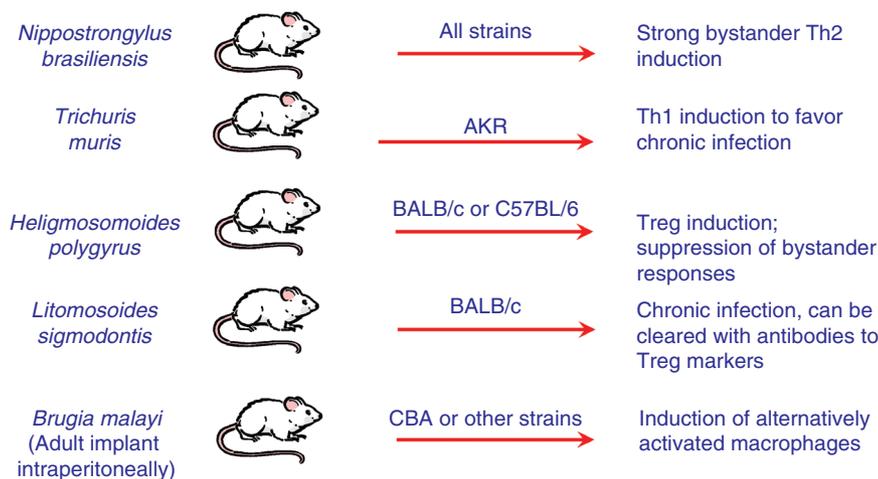


Fig. 2. Murine models for immune modulation in nematode infection.

develop a chronic infection with strong Th1 responses (31). Manipulation of mice with exogenous cytokines or anti-cytokine antibodies will coordinately switch Th phenotype and susceptibility in accordance with this principle (32).

The *T. muris* system offers a clear paradigm that Th2 responses protect against infection, strongly supported by data from the other gastrointestinal nematode species (33). *T. muris*, however, is the only nematode studied that (in susceptible mice) avoids generating a Th2 response altogether. Why have many other gut helminths not adopted a similar evasive strategy? One possibility is that in many circumstances the downregulation of type 1 inflammatory mediators is more beneficial to the parasite. For example, epithelial tissue disruption exposes the host not only to parasite-derived factors but also to inflammatory microbial molecules derived from the normal host flora. It is possible that many gastrointestinal helminths need to suppress inflammation of the gut micro-environment caused by microbial-derived mediators. Indeed, it is known that the presence of gastrointestinal nematode infection will inhibit inflammatory bowel disease in humans (34) and experimental colitis in mice (35). There is also an observable threshold effect: most hosts tolerate low levels of helminth infection, and Th2 responses may not reach the level where effector mechanisms are triggered. For long-term infection, the maintenance of normal gut function is crucial to parasite survival, and a Th2 bias that downregulates type 1 inflammation in the intestinal environment is likely to be of benefit to the parasite in most cases.

Th2 induction

The potency of helminth induction of Th2 bias is firmly established, with all the classic type 2 cytokine and isotype markers amplified in nearly all infections (2, 3). Early work had reported that IgE responses to bystander antigens could be amplified in animals with a concurrent *N. brasiliensis* infection (36) or in animals that were co-immunized with *Ascaris*-secreted antigens (37). A cytokine profile of *S. mansoni*-infected animals showed that the phenotype of the response to bystander antigens was indeed driven in the Th2 direction (38). Thus, both the intensity and the scope of Th2 bias induced by helminths are remarkable. How is such a one-sided response mode governed?

N. brasiliensis is perhaps the classic Th2-driving infection in rodents (26–29). We have developed this system by culturing adult worms and collecting the glycoproteins they secrete *in vitro*: the *N. brasiliensis* excretory-secretory (NES) products (Fig. 3A). NES drives Th2-biased responses without requiring live infection of mice, and the activity is abolished by either heat or protease treatment (39). NES acts as a Th2-promoting adjuvant on bystander antigens, as co-administration of soluble NES with hen egg lysozyme (HEL) provokes a Th2 response specific to the coincident HEL antigen (39). Moreover, NES immunization of mice generates a Th2 immune response whether administered as a soluble protein or in complete Freund’s adjuvant (CFA), which normally favors the development of Th1 immune responses. The ability of NES to generate

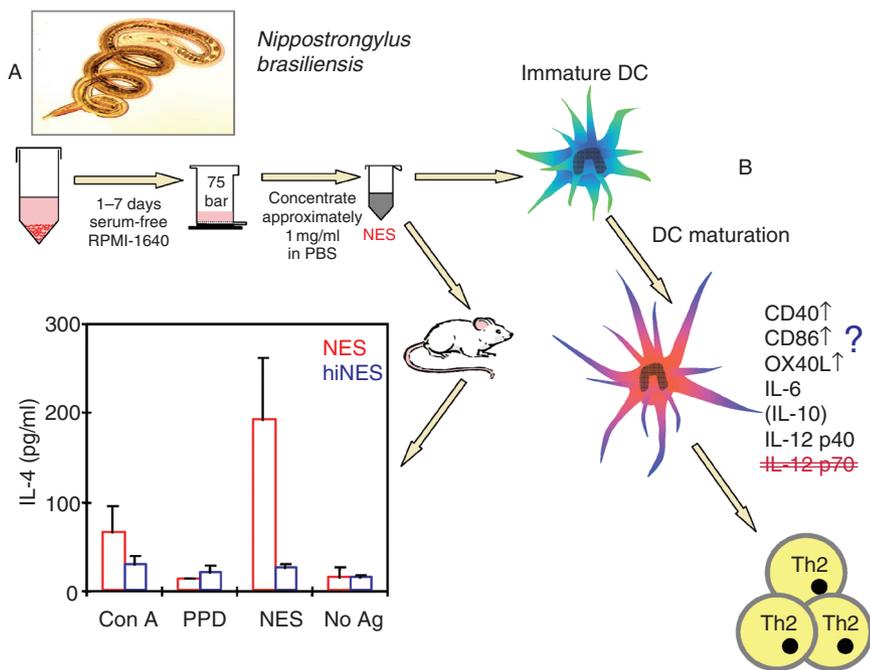


Fig. 3. T-helper 2 cell (Th2) induction by *Nippostrongylus*. (A) Isolation and Th2 induction by *Nippostrongylus* excretory-secretory (NES) antigen (40). (B) Model for Th2 induction by NES-pulsed dendritic cells (DCs) (49). IL, interleukin; PBS, phosphate-buffered saline.

a strong Th2 response when co-administered with CFA is clear evidence that the generation of Th2 immune responses by helminth parasites is not simply due to a 'default' in the absence of Th1-oriented stimulation.

This system allowed us to dissect some of the key features of Th2 induction. Responses were comparable in wildtype and in major histocompatibility complex (MHC) class I-deficient ($\beta 2$ -microglobulin^{-/-}) mice, as well as in IL-5 knockout (KO) animals and B-cell-deficient (μ MT) mice (40). In terms of the induction of Th2, these studies indicated that eosinophils, B cells, or natural killer T cells do not play a critical role in the initiation of the Th2 pathway. Moreover, IL-4 itself is not essential for the early type 2 response to NES; mice deficient in the IL-4R are able to mount an IL-4 response in the CD4⁺ population, arguing that other signals are sufficient for Th2 differentiation to begin (Balic and Maizels, unpublished observation).

NES shares some common features with Th2-inducing materials from other helminths, but there are also many critical differences, which lead us to suggest that different parasites have evolved unique mechanisms to achieve a similar effect. The most obvious distinction is the heat lability and protease sensitivity of NES, compared to the glycan moieties associated with Th2 stimulation from filarial nematodes (5) and schistosomes (41). However, even in the schistosome egg, not all Th2-driving capacity resides in the glycans; an important protein, active as a non-glycosylated recombinant, cross-links mast cells to release IL-4 (42). Similarly, immunization of mice with non-glycosylated recombinant antigen (NPA-1) from the filaria *Diriofilaria immitis* results in the production of IL-4, IL-10, and IgE (43). The wide diversity of Th2-stimulating molecules implies two extremely important points: different helminths have independently evolved a variety of pathways for Th2 stimulation, and this propensity is likely to have been positively selected to confer a biological advantage to each parasite species.

Dendritic cell activation for Th2

Th2 induction, like all adaptive responses, requires the initial involvement of dendritic cells (DCs). For Th1 responses, the paradigm has become firmly established that DCs presenting bacterial or protozoal antigens are prompted to release IL-12 and upregulate key costimulator proteins such as CD80 and CD86 (44). How DCs elicit Th2 responses is more controversial: is there a mirror-image signature of cytokines and surface ligands that DCs express to stimulate Th2 differentiation? Or is

the very lack of Th1-driving molecules sufficient, by default, to result in a Th2 response (45)?

Several experimental systems show that Th2 induction can be reproduced simply by exposing DCs to helminth products *in vitro*, and then transferring the pulsed DCs into live recipients. These products include the filaria *Acanthocheilonema viteae* ES-62 (46), *S. mansoni* soluble egg antigen (SEA) (47), the schistosome-associated glycan lacto-N-fucopentaose III (48), as well as the *N. brasiliensis* NES antigens (49). Notably, in the first three of these studies, no new phenotype of helminth antigen-stimulated DCs could be discerned, nor was any significant increase in the cytokines IL-4, IL-10 (which would account for inhibition of Th1) seen. This evidence supports the hypothesis that the Th2 immune response is a default mode that occurs when DCs fail to mature.

Results with NES in our own laboratory argue, however, that DCs are indeed activated to induce Th2 responses, although the activation profile is more subtle than that resulting from lipopolysaccharide (LPS) exposure (49). Following injection with NES-pulsed bone marrow-derived DCs, mice are efficiently primed for a dominant Th2 response. Heat-inactivated NES (hiNES) is unable either to drive Th2 responses *in vivo* or to stimulate DCs for Th2 induction. NES, but not hiNES, upregulated DC markers associated with Th2 promotion (CD86 and OX40L), while CD80 and MHC class II levels were unchanged. OX40L has previously been found to be required for optimal Th2 induction in the response to *H. polygyrus* (50).

Moreover, NES-exposed DCs produced IL-6 and IL-12p40 but not IL-12p70. Most critically, the IL-12p70 response of DCs to LPS was abolished by prior incubation with NES. These data directly contradict the default hypothesis by showing that NES actively matures DCs, upregulates CD86 and OX40L, blocks IL-12p70 production, and switches the cytokine profile to release IL-6 (Fig. 3B). While the respective roles of CD86, OX40L, and IL-6 in the generation of Th2 responses remain unresolved, the suppression of type 1 inflammatory mediators by antigen-presenting cell (APC) populations is emerging as a common theme of helminth infection. Macrophages from mice infected with *Toxocara canis* produce normal levels of IL-6 but exhibit suppressed IL-12 and TNF- α responses to LPS stimulation (51). Similar effects, although also suppression of IL-6, are seen in murine macrophages exposed to ES-62, a purified and highly potent immunomodulator derived from *A. viteae* (52). More recently, Agrawal and colleagues (53) have demonstrated that stimulation of human monocyte derived DCs with schistosome SEA results in high-level production of IL-6 but suppressed IL-12p70 production due to the modulation of the

transcription factor c-Fos. Blockade of c-Fos by short interfering RNA restored IL-12p70 production in SEA-stimulated DCs.

Three examples of a dominant Th2 response cast further doubt on the default hypothesis. The first 6 weeks of response in murine schistosomiasis is dominated by Th1 responses to larval antigens. Only subsequently, with egg release, is there a switch to Th2 (54), although the Th1-stimulating products are presumably still present. A similar switch from early Th1 to later Th2 is seen in mice infected with *Taenia crassiceps*, the cestode which causes cyticercosis (55). A different contradiction exists in filariasis, as the larval and adult stages stimulate a heavily biased Th2 response, but the microfilariae, if injected on their own, drive Th1 differentiation (56). However, in the presence of adult worms, no Th1 response is seen. Perhaps the clearest contradiction of the default hypothesis for Th2 induction is provided by recent work in which DCs co-exposed to SEA and the Th1-inducing *Propionibacterium acnes*, when injected into naïve mice, elicit a Th2 response to the SEA and a Th1 response to the bacteria (59). Interestingly, exposure to SEA reduced the IL-12 response to *P. acnes*, a finding similar to those discussed above with respect to nematode Th2 induction.

A particular enigma in filariasis is the presence of the endosymbiotic bacterium *Wolbachia*, which has demonstrable pro-inflammatory (Th1) LPS-like activity (58). In human infection, *Wolbachia* products may be most important immediately following drug clearance, where the release of bacteria from dead worms may correlate with high levels of serum cytokines and adverse effects of therapy (59). Likewise, in onchocerciasis, the presence of *Wolbachia* exacerbates the inflammatory reaction to microfilariae in the eye (60). A wider question is why, if prominent bacterial stimulation occurs during asymptomatic infection with live worms, the resulting phenotype is still so Th2 dominated? Treatment *ex vivo* of macrophages recruited during filarial infection with LPS or IFN- γ converts them from type 2 (alternatively activated) to a classically activated phenotype (Nair and Allen, unpublished observation). However, *in vivo*, in the presence of *Wolbachia*-containing adult worms and microfilariae, the macrophage activation state remains profoundly type 2 (61, 62). This finding suggests that the LPS-like activity is not sufficient under normal parasite turnover to alter macrophage phenotype. Moreover, we note that the type 2 response is actively maintained *in vivo* regardless of the pro-inflammatory *Wolbachia*, demonstrating that Th2 phenotype does not arise simply in default of pro-Th1 signaling.

While DCs provide the first impetus to the immune response, they are not the only innate cell type able to influence the direction and outcome of the adaptive immunity. There is an increasing appreciation that mast cells and granulocytes (baso-

phils, eosinophils, and neutrophils) can react directly to the presence of helminth parasites, by generating cytokines and chemokines, prior to their entry into the scene as producers of toxic and inflammatory granule proteins. Recent work by Locksley and colleagues (63) has demonstrated that during primary infection of mice with *N. brasiliensis*, IL-4-producing eosinophils and basophils are actively recruited to the site of larval migration in the lung (63). However, eosinophils are only able to degranulate in mice that contain intact CD4⁺ T-cell responses (64). At this point, it is unknown whether these eosinophils require T-cell-derived signals to release regulatory cytokines and chemokines, but it does raise the intriguing possibility that granulocytes may help shape the outcome of the developing immune response, rather than serve simply as late-stage effector cells involved with expulsion of the parasite. Furthermore, if these innate effector cells switch from early regulatory to subsequent aggressive capacity, it is possible that, in the final phases of the response, they again act as regulators to dampen adaptive immunity.

Th2: protective or ineffective?

Although induction of a potent type 2 immune response is characteristic of helminth infection, the outcome for parasite and host of this highly skewed response is not always apparent. A long-running debate in parasite immunology has been whether the Th2 arm of immunity is responsible for the elimination of helminth parasites (65–67). In gut nematode infections, the case for Th2-dependent immunity is clear-cut (68, 69): IL-3/IL-9-stimulated mast cells (70, 71), IL-4R-stimulated intestinal muscle cells (72), and IL-13-stimulated epithelial goblet cells are each essential for resistance to different gastrointestinal parasites. One of the powerful lessons from these studies is that although an array of Th2-driven effector mechanisms is mobilized, only particular components prove to be effective against any one species (73). This conclusion is borne out across the spectrum of helminths in general; not only do the most effective pathways vary for each species but also protective mechanisms differ at each stage of the life cycle as parasites migrate through different tissues and undergo developmental changes in structure and biology.

Beyond the gastrointestinal locale, our understanding of protective mechanisms is less conclusive, because Th2 responses often coincide with stable infections impervious to immune attack and because of the variety of tissue sites that may accommodate the parasites. Perhaps the clearest picture is of immunity against incoming nematode larvae, both from species which

remain in the tissues (such as the filarial worms) and those which migrate onward to the intestinal tract to mature. A strong consensus has emerged that killing of migratory larvae is most effectively achieved by eosinophils (74–77). With respect to successful experimental (78–81) and commercial (82) irradiation-attenuated vaccines, which stimulate strong protective responses against a challenge infection, good evidence exists to argue that both eosinophils and antibodies are required (74, 83). Despite conclusive data on tissue larval killing by eosinophils, these cells have little apparent efficacy against adult worms in the tissues or in the gut. Remarkably, we still fail to understand how the adult stage of filarial parasites, schistosomes – or for that matter any helminth – is physically killed by the immune system *in vivo*.

Identifying the effector mechanisms in immunity to filariasis is a particular challenge, combining the issues of stage-specificity, different tissue tropisms, and a strong influence of host genetics. Work in filariasis was constrained in the absence of a murine model to investigate both the immunology and the natural migration of filarial parasites. The discovery that *Litomosoides sigmodontis* can produce patent infections in BALB/c mice (84, 85) has removed this impediment. Entering at the mite vector bite site, *L. sigmodontis* stage 3 larvae (L3) migrate via the lymphatics, reaching the thoracic cavity by 6 days after infection (Fig. 4). There they mature, and by day 55 microfilariae are found in peripheral blood. In contrast, in C57BL/6 mice, the parasite fails to reach sexual maturity, and all larvae molt to the L4 stage and some to the adult stage, although significantly retarded in growth (86). The ability to establish early infection in both resistant and susceptible hosts may be due to the profound downregulatory capacity of incoming larvae, potentially a feature of both filarial L3 (87, 88) and

Schistosome cercariae (89). An interesting distinction also exists between these studies of primary infection and those on immunity induced by vaccination or multiple infection; in the latter cases, the primary targets are larvae before or during the molt to L4 (83, 90–92). Perhaps, if the immune system fails to kill incoming larvae promptly, parasites have the opportunity to induce immunoregulation, which delays or prevents the expression of immunity in genetically resistant or susceptible strains, respectively.

The *L. sigmodontis* model has also allowed us to unravel potential effector pathways in filariasis at the level of the cytokine involvement. This investigation may provide a useful paradigm for understanding how the immune system can eradicate a parasite that has already overcome hostile conditions for long enough to mature to adulthood and patency. Using this model system, it has become possible to ask the following: what is the role of the Th2-mediated response in resistance to filarial infection, and what is its role at different stages of the parasite life cycle? To address the first question, we infected IL-4 deficient mice on the resistant C57BL/6 background. These mice had infection profiles indistinguishable from the susceptible BALB/c strain demonstrating that as for gastrointestinal nematodes, IL-4 is a critical determinant of resistance (93). Interestingly, IL-4 deficiency on the BALB/c background has little effect on adult recovery (93, 94). We hypothesized that on this background, IL-13 may be compensating for the absence of IL-4, and that in the absence of both cytokines, we would generate a ‘super-susceptible’ mouse. Surprisingly, infection of IL-4 receptor (IL-4R α)-deficient BALB/c mice (which cannot respond to either IL-4 or IL-13) rather than resulting in enhanced parasite survival, led to accelerated death of the adult stage (Nair and Allen, unpublished observation).

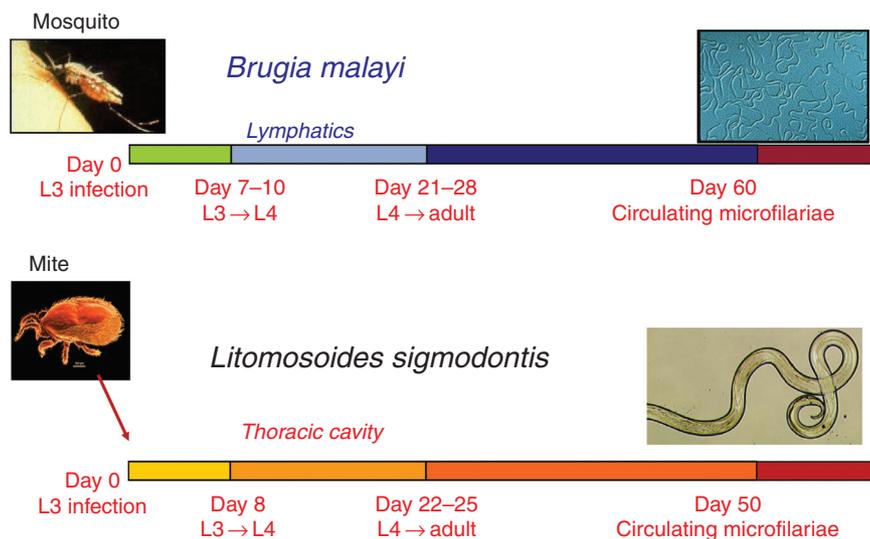


Fig. 4. New mouse model of filariasis, *Litomosoides sigmodontis*.

When we looked at the phenotype of these mice, they had switched to a type 1 response both at the level of T-cell cytokines and at the level of macrophage activation. These data, suggesting that the adult parasite can be killed by type 1 inflammatory response, are consistent with the findings of Hoerauf and colleagues (83, 95, 96), who have demonstrated a role for IFN- γ along with IL-5 in the killing of adult *L. sigmodontis*.

Our understanding of what mediates control of the circulating microfilarial (MF) stage is perhaps the most advanced. IL-4 receptor KO BALB/c mice infected with *L. sigmodontis* exhibit distinct mechanisms for control of the adult versus the MF stage. While the absence of IL-4/13 signaling led to more rapid adult killing, it dramatically enhanced MF survival with enormously high levels of circulating MF in the KOs relative to controls, even after adult parasites had been cleared (Nair and Allen, unpublished observation). The importance of IL-4 and IL-13 in controlling MF is consistent with other studies in *L. sigmodontis* (96) as well as *Brugia pahangi* (94). Mechanistically, this is supported by models where microfilariae are injected directly into the bloodstream, which demonstrate that despite the ability of nitric oxide (NO) to kill microfilariae *in vitro*, neither IFN- γ nor NO is an important effector *in vivo* (97, 98). The data suggest that IL-4 and IL-13 are involved in the clearance of microfilariae from the blood stream, perhaps through mediating antibody-dependent cell-mediated cytotoxicity (ADCC) (99), with additional effects of IL-4 on adult worm fecundity (94, 96), although the mechanism for this effect is not known.

Type 2 responses are thus a critical determinant of the outcome of filarial infection but with very different dynamics depending on the stage of the parasite and strain of the host. Although a role for IL-4 and IL-13 in microfilaria control seems to be a consistent finding, killing of the adult stage may require IL-5 as well as the type 1 cytokine IFN- γ (95, 96). Interestingly, it is IFN- γ and IL-5 that are downregulated in humans with active filarial infection, supporting the possibilities that these cytokines are active against the human filariae as well. IFN- γ may be necessary because of its well-known pro-inflammatory functions. Alternatively, IFN- γ was recently found to downregulate the IL-13 α decoy receptor, thus effectively increasing the levels of available IL-13 (100), which may also be a critical factor in the anti-parasite response. We do not as yet have a good appreciation of the interaction or synergy between both adaptive and innate cells and the nominally Th1 cytokines in the context of a type 2-dominated immune response. For example, mice lacking TNF- α fail to generate a protective Th2 response to *Trichuris* (101), but

this scenario has yet to be investigated in other helminth infections.

The central conundrum in helminth diseases remains that infections such as filariasis and schistosomiasis survive well in humans with dominant Th2 responses. This pattern is replicated in *L. sigmodontis*-infected BALB/c mice, in which parasites establish patent infection in the face of strong type 2 responses (93). Why then are these responses ineffective? First, Th2 cells cannot kill parasites on their own; they rely on cytokine-mediated activation of innate effector cells (as evident in the intestinal setting). Second, Th2 cells are not uniform in either their own activation state or the cocktail of cytokines that they produce (102, 103). We suggest that in chronic helminth infection, the production of key Th2 effector cytokines (particularly IL-5 and IL-13) are downregulated, although the Th2 regulatory cytokines (IL-4 and IL-10) continue to be produced, lending a 'Th2 signature' to the overall response.

How and why do Th2 cells become modified or deactivated? Two possibilities exist. First, the Th2 population may autoregulate, for example producing late-stage inhibitors which feedback to rein in the response because of its potential to cause damaging fibrosis (104). An example of this model is the decoy receptor for IL-13, a soluble competitor for cell-surface IL-13R that is able to neutralize Th2 inflammation and pathology in schistosomiasis (104, 105). Second, Th2 cells may be conditioned by an extraneous population, such as the regulatory T cell.

Regulatory T cells

The field of infectious disease immunology is at an exciting intersection between new general concepts in immune regulation (106–108) and explicit evidence that parasites stimulate suppressive T-cell populations, termed regulatory T cells (Tregs) (109). Tregs produce downmodulatory cytokines (IL-10 and TGF- β) that switch off inflammatory and protective immune responses and interfere with effector T-cell activation in a contact-dependent manner. Understanding of Treg genesis and activity is based primarily on the control of pathogenic autoimmunity by CD4⁺CD25⁺ T cells selected on self-antigen in the thymus. More recently, however, it has become apparent that Tregs can be induced to regulate responses to exogenous antigens, whether from innocuous commensals or infective pathogens. Evidence from a variety of infectious systems argues for Treg control in viral (110), bacterial (111, 112), and protozoal (109, 113) infections. In some cases, Tregs appear to prolong pathogen survival, while in others they downregulate potentially pathogenic immune

responses. A clear example of the latter is in mice infected with *S. mansoni*, in which CD25⁺ T cells are the major source of the IL-10 essential to protect animals from fatal immunopathogenic responses to eggs in the liver (18).

Are Tregs responsible for the immune suppression in filariasis? There are numerous reports linking human schistosome and filarial infections (onchocerciasis and lymphatic filariasis) with raised IL-10 and TGF- β production by peripheral lymphocytes (20, 21). Neutralization of these cytokines in human peripheral blood lymphocyte (PBL) cultures has been shown to reverse antigen responsiveness toward filarial antigens (19, 114). More recently, cloned T cells with a regulatory phenotype have been isolated from onchocerciasis patients (115, 116). As discussed above, filarial suppression is predominantly antigen specific but shows some ‘spillover’ toward bystander responses, consistent with the ability of antigen-specific regulatory cells to downmodulate both cognate and non-cognate responses through cytokines such as IL-10 and TGF- β . The resonance between Treg activity in mouse model systems and the observations in human helminth infections led us to investigate the role of Tregs in experimental nematode infections (Taylor M, Wilson M, Harris A, Malore E, Allen J & Maizels RM, unpublished observations).

We approached this question by analyzing the immunological events in the recently developed mouse model system for filariasis, *L. sigmodontis* (Fig. 4). In this system, potentially protective Th2 responses (IL-4 and IL-5) are evoked in susceptible mouse strains from an early stage, but no protective effect is apparent. The Th2 response is counterpointed by an early increase in *Foxp3* mRNA expression in the draining lymph nodes (LNs) (Taylor, Harris, and Maizels, unpublished observation), signifying upregulation of this key transcription factor associated with Treg function (117). There is also increased production of the regulatory cytokines IL-10 and TGF- β

throughout infection. Following adult worm establishment and MF entry into the bloodstream, a more profound suppression takes hold. CD4⁺ T cells show increased expression of the regulatory markers cytotoxic T-lymphocyte antigen-4 (CTLA-4) and glucocorticoid-induced tolerance receptor (GITR), while parasite antigen-specific proliferative and IL-5 responses decrease (Fig. 5A).

To test directly whether Tregs are prolonging infection, we administered antibodies to regulatory T-cell-surface markers in infected mice. We selected CD25 (the IL-2R α chain) as one target, which is constitutively expressed by natural Tregs (118). The second target chosen was GITR, first reported to be a Treg surface protein (119) but now understood to be expressed as a costimulator on effector T cells (120, 121). Neither antibody alone altered the worm burden in infected mice, but the combination of both antibodies evoked clearance (73% reduction) of adult *L. sigmodontis* from the thoracic cavity (Taylor et al., unpublished).

The enhanced parasite clearance was associated with increased immune responsiveness toward parasite antigens, suggesting that we had indeed neutralized regulatory activity. In particular, IL-5 production increased, an interesting finding as this is one of the cytokines known to be downregulated during chronic human infection. In terms of the *Litomosoides* model, IL-5 is also a key cytokine involved in the killing of both adult *L. sigmodontis* and its infective L3 stage (78, 96).

IL-10 also rose after Treg ablation, suggesting that it is not the key mediator of suppression during *L. sigmodontis* infection. In agreement, attempts to abolish Treg activity using antibodies against the IL-10R *in vivo* and *in vitro* failed to influence either parasite recoveries or proliferative responses. This lack of effect of IL-10 may be related to the Th2 nature of resistance toward filarial worms; while IL-10 is the crucial regulator produced by Tregs during a Th1-dependent *Leishmania*

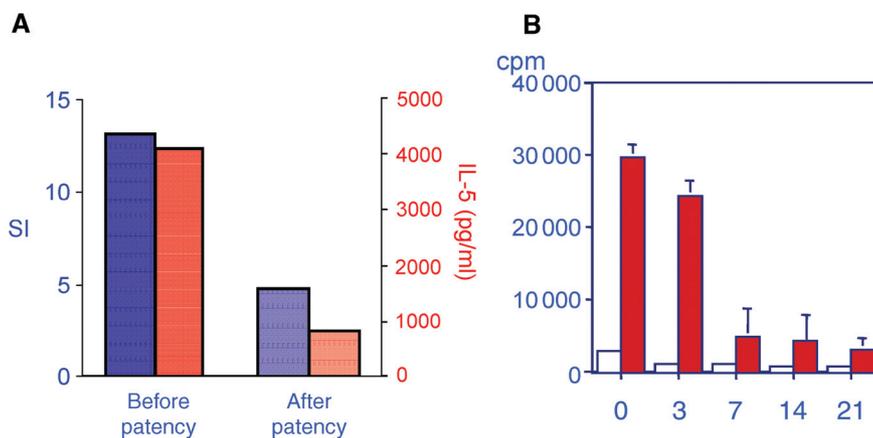


Fig. 5. Both T cells and macrophages are altered in mouse filarial infection. (A) T cells from *Litomosoides*-infected mice fail to proliferate in response to parasite antigens (Taylor et al., unpublished). (B) Macrophages from *Brugia malayi*-infected mice suppress T-cell proliferation.

major infection (109), its role in regulating Th2 responses is less clear-cut, as it can promote Th2-dependent immunity toward helminth parasites (122, 123). In contrast to the adult filariae, microfilariae do induce a Th1 response with IFN- γ production (56) and are cleared more rapidly in IL-10^{-/-} mice (124). The immunoregulatory action of IL-10 may therefore be specific to the microfilaraemic stages of filarial infection, whereas our studies in the *Litomosoides* model have concerned the earlier phase of adult establishment. Interestingly, in humans, IL-10-mediated suppression is associated with microfilaraemic individuals with long-term chronic infections. In contrast, neutralization of IL-10 does not affect immune reactivity in individuals only recently exposed to filarial infection (19, 114). The regulatory role of IL-10 may therefore be more important during long-term chronicity and the control of microfilaraemia, in distinction to its role early in infection where it is necessary for induction of a strong Th2 response.

For the first time, it has been demonstrated that nullifying regulatory T-cell activity can ‘cure’ chronic helminth parasite infection by allowing the immune system to operate at its full potential. This supports the model that during chronic infection, the host immune system fully recognizes parasite antigens but is hampered from reacting to them effectively by the activity of parasite-specific suppressor/regulatory mechanisms (Fig. 6). Thus, immunity requires the removal of suppressive cells, if it is to be expressed. This requirement is of particular consideration when trying to stimulate vaccine-elicited immunity in individuals who have been chronically exposed to the parasite and who may be unable to respond to the vaccine. Future immunological therapy to cure chronic infections may

need to target regulatory T cells, and by de-activating them release the underlying potential of the host immune system to destroy parasites.

The conditioned Th2 cell

Our data from the *Litomosoides* model, which demonstrated a role for CD25⁺ Tregs, also suggested that the hyporesponsive phenotype outlasted removal of Tregs. This led us to propose the concept of ‘conditioned Th2’. While the CD4⁺ T cells isolated at patency from the site of infection were hyporesponsive to parasite antigens, they did not demonstrate a suppressive phenotype during *in vitro* stimulation nor did they increase their expression of *Foxp3*. These cells therefore appear to be effector cells that have been turned off or ‘conditioned’ toward a hyporesponsive phenotype, rather than an expanded Treg population. This hyporesponsive or conditioned phenotype was associated with a dramatic upregulation of the costimulatory molecule GITR and the co-inhibitory molecule CTLA-4 (approximately 70% of the population becoming CTLA-4⁺GITR^{high}) (Taylor et al., unpublished). The requirement for treatment with both anti-CD25 and anti-GITR can now be interpreted as a two-stage process: depletion of CD25⁺ cells removes the regulatory population, while ligation of GITR in the absence of regulation reactivates the anti-parasite response preventing or reversing conditioning.

The expression of high levels of the co-inhibitory molecule CTLA-4 by *L. sigmodontis*-conditioned CD4⁺ T cells presents a prime candidate for mediating their hyporesponsive phenotype. Among the precedents for this hypothesis are the findings that

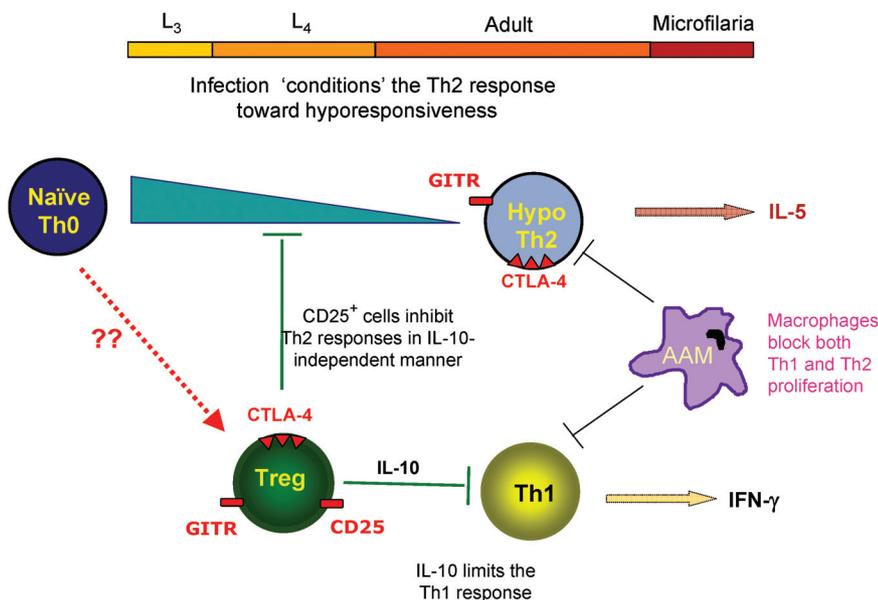


Fig. 6. Hypothesis for immune regulation in *Litomosoides sigmodontis* infection. CTLA-4, cytotoxic T-lymphocyte antigen-4; GITR, glucocorticoid-induced tolerance receptor; IFN- γ , interferon- γ ; IL-5, interleukin-5.

CTLA-4 is responsible for maintaining the anergic phenotype of T cells after oral tolerance (125), and it plays a critical role in inhibiting Th2 responses (126). When studied in relation to a Th2-inducing parasite, CTLA-4 blockade during *N. brasiliensis* infection resulted in enhanced parasite-specific immunity, stronger Th2 cytokine production, and diminished parasite numbers (127). As well as directly inhibiting T-cell activation through the T-cell receptor, CTLA-4 has also been found to act on DCs by reverse signaling through CD80/CD86. This signal induces them to increase indoleamine 2,3-dioxygenase, which in turn leads to the inhibition of T-cell responses (128). During *L. sigmodontis* infection, therefore, expression of CTLA-4 may not just downregulate the T cells themselves, but through its action on APCs may extend the hyporesponsive phenotype to non-conditioned T cells. In such a situation, the balance of expression of co-inhibitory CTLA-4 and costimulatory molecules, such as GITR or CD28, would be crucial in determining the host's ability to respond effectively to infection.

Similarities to the conditioned Th2 response seen in the *Litomosoides* model can be found in the human context (Fig. 7). PBLs taken from individuals exposed to chronic helminth infections have an anergic phenotype, showing impaired signal transduction after T-cell activation (129). In this study and in studies on human filarial infection (130), T-cell unresponsiveness was associated with increased CTLA-4 expression and could be reversed through its blockade. A further parallel is seen in comparison with allergic reactivity in atopic humans, who exhibit a Th2 effector response (IgE and eosinophils) to innocuous antigens. Desensitization (by administering minute quantities of allergen) alters the phenotype, not from Th2 to Th1, but within the Th2 compartment. A 'modified Th2' state is observed (131), in which IgG4 titers rise, IgE falls, and

patient allergy is diminished. Interestingly, the IgG4/IgE ratio is modulated by IL-10 (134). Moreover, in asymptomatic 'regulated' helminth infection, IgG4 is the dominant isotype, but levels of IgG4 decline sharply after chemotherapy (135), arguing that the pressure from the presence of parasites, perhaps acting through high IL-10, maintains unusually high IgG4 levels.

Alternative activation of macrophages

The type 1/type 2 dichotomy is reflected most dramatically at the level of the disparate effector pathways these cytokines induce. Th1-cell effector function has traditionally been associated with the activation of macrophages by IFN- γ to destroy intracellular pathogens while Th2-driven effectors included granulocytic cells, such as eosinophils, mast cells, basophils, as well as cytophilic antibody isotypes. These together can mediate ADCC and the killing of extracellular pathogens. One largely ignored feature of this paradigm is that macrophages often outnumber the granulocytes in a Th2-mediated cellular infiltrate (134, 135). What role these macrophages play in the type 2 response is still not evident. They are considered to be alternatively activated macrophages (AAM Φ s) because they exhibit an activated surface marker phenotype but are diametrically opposed to 'classically activated' macrophages, as they do not upregulate inducible NO synthase but instead the cross-regulatory enzyme arginase (136). This phenotype was originally described *in vitro*, but we and others have demonstrated that it is also present *in vivo* in parasite infection (61, 137, 139). Data now suggest that AAM Φ s are active in three functional categories (Fig. 8). First, as an important downregulatory cell, they may suppress the

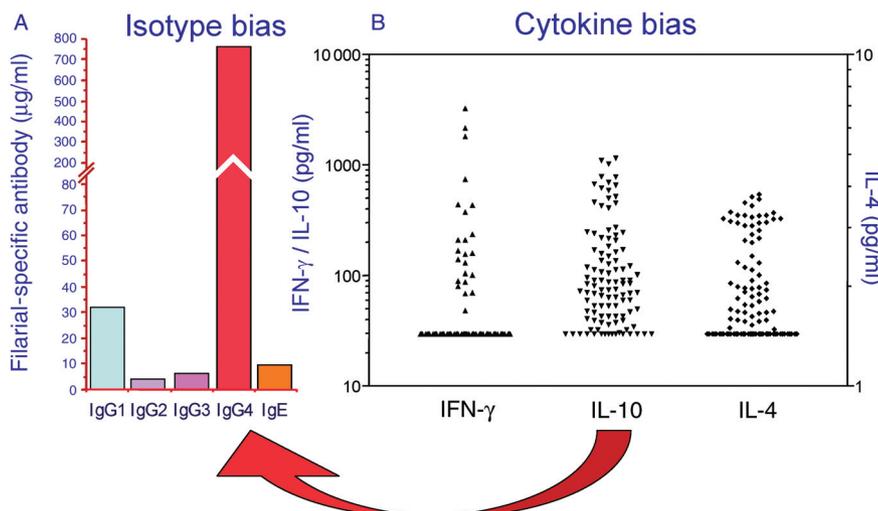


Fig. 7. Not all T-helper 2 cell (Th2) responses are the same: the modified Th2 phenotype. (A) The dominant immunoglobulin G4 (IgG4) response to filarial antigens in asymptomatic *Brugia malayi*-infected patients [adapted from (261)]. (B) The balance of cytokines in the filarial response (data from M. Yazdanbakhsh). Ig, immunoglobulin; IFN- γ , interferon- γ ; IL-5, interleukin-5.

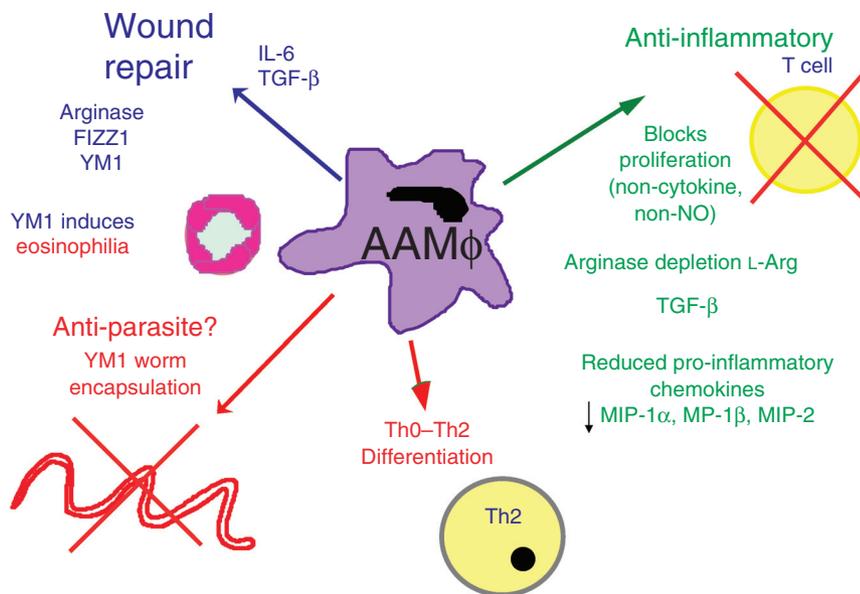


Fig. 8. Functions of the alternatively activated macrophages (AAMΦs). IgE, immunoglobulin E; IL-5, interleukin-5; NO, nitric oxide; TGF-β, transforming growth factor-β; Th2, T-helper 2 cell.

inflammatory response to the parasite. Second, their recruitment in high numbers suggests that they are important effector cells, releasing molecules that target extracellular helminths and further promote the Th2 immune response. Finally, they may be required to repair the damage caused by large extracellular helminths.

The evidence that AAMΦs are an important suppressor cell is based in part on the profoundly anti-proliferative activity of macrophages in type 2 settings. Human studies have demonstrated that adherent phagocytic mononuclear cells mediate similar suppression in filariasis (139) as well as schistosomiasis (140). Mice implanted with adult *Brugia malayi* generate large numbers of AAMΦs (141), which suppress the proliferation of a range of target cells through a contact-dependent mechanism (62). They arise within 7 days of adult *B. malayi* transplantation into the peritoneal cavity (Fig. 5B) but are absent if dead parasites are transferred (141). Similar cells have also been described in chronic cestode infection (137). Likewise, macrophages in the schistosome egg granuloma, an intensely type 2 environment, have been shown to be down-regulatory (142), while injection of schistosome sugars induces a Gr1⁺ cell that blocks proliferation in a cell contact-dependent manner (143). These latter cells are phenotypically related to the immature myeloid cells that infiltrate tumors, sharing properties such as arginase production and suppression in an NO-independent manner (144, 145).

The mechanism of suppression remains unknown, but IL-4 induces some anti-proliferative activity *in vitro* (146, 147) and more so *in vivo* (147). Arginase is produced by AAMΦs and may create a suppressive environment by depleting arginine

from the surrounding environment (144). That this suppressive phenotype is important in helminth infection *in vivo* is supported by functional analyses of LN cells from *L. sigmodontis*-infected BALB/c mice, in which T-cell proliferative responses decrease as infection progresses. Proliferation is restored if CD4⁺ T cells from infected animals are cultured with fresh APCs from naïve mice, but not if co-cultured with whole LN cells or F4/80⁺ purified macrophages from infected mice (Taylor, Allen, and Maizels, unpublished observations). As suppressive cells are also found alongside the conditioned CD4⁺ T cells at the site of *L. sigmodontis* infection, at least two independent levels of regulation are present once infection reaches patency.

In mice, suppression is strain independent; anti-proliferative macrophages occur in every mouse strain tested including BALB/c, CBA, and C57BL/6. The anti-proliferative phenotype is highly dependent on type 2 cytokines, but the requirement for IL-4 versus IL-13 does vary between strains. IL-4 deficiency is sufficient to abolish the anti-proliferative phenotype in C57BL/6 mice but not in BALB/c, where removal of IL-13 activity is also required (Nair, Gallagher, and Allen, unpublished observations). Regardless, macrophages recruited in nematode infection suppress the proliferation of neighboring cells only in the context of a full-blown Th2 environment. This finding led to our belief that we were observing an *in vivo* form of AAMΦs, in a mouse model system highly appropriate for delineating the function of these macrophages.

We surmised that a snapshot of the genes most abundantly expressed by macrophages recruited in nematode infection would illuminate the primary function of these cells. Using

the *Brugia* implant model, we assessed gene expression in suppressive AAMΦs (61). Surprisingly, the two highly abundant genes *Ym1* and *Fizz* were represented by 10 and 2% of cDNA, respectively, but were novel genes with no known function. We have since found that expression of these proteins is a near-universal feature of helminth infection, focused at sites of parasite migration and residence in both acute (*N. brasiliensis*) and chronic (*L. sigmodontis*) nematode infection as well as in the lungs of schistosome-infected mice (Nair, Taylor, Allen, unpublished). Others have found these products in the context of cestode infection (137), and they are also upregulated in the Th2-dominated chronic stages of protozoan infection (148). The rapidly expanding literature on *Ym1* and *Fizz* in non-infectious contexts is helping to generate a picture of their function, although many questions remain to be answered.

The gene expression profile of these macrophages draws intriguing links to immune responses during allergy, in particular asthma, in which both *Fizz1* and *Ym1* are induced, suggesting that there may be a common phenomenon of dysregulated Th2 inflammation. *Fizz1* was first identified as an abundant protein secreted from inflamed alveolar epithelium (149), and it more recently was implicated in stimulating myofibroblast differentiation and fibrosis during pulmonary inflammation (145). Although *Fizz1* expression has not been reported in alveolar macrophages, *Ym1* is expressed in this cell type during lung development (150) and is upregulated in allergy (151). We have also observed increased secretion of both proteins in the lungs of transgenic mice overexpressing IL-4 and mice infected with *N. brasiliensis*. Alveolar macrophages also suppress T-cell proliferation and the production of certain cytokines (152–154), drawing links to macrophages recruited during nematode infection. In the alveoli, the downregulation of potentially pathogenic T-cell responses against irrelevant antigens is critical, and helminths may have evolved to stimulate or mimic similar downregulatory interactions to promote their own survival.

Whether *Fizz1* and *Ym1* are beneficial or exacerbate disease during helminth infection is unclear. In infection of resistant mice with *L. sigmodontis*, the dying worms are often encapsulated in granulomas consisting predominantly of macrophages and eosinophils (78, 93). As *Ym1* has eosinophil chemotactic activity (155), its secretion by macrophages may lead to the recruitment of eosinophils able to kill parasites. Granuloma formation could be mediated by the macrophage through the combined action of arginase, *Fizz*, and *Ym1*, in an attempt to encapsulate the parasites. Through its ability to influence the action of nerve growth factor (NGF) (149), *Fizz1* could also have an important impact on the cell types recruited, as NGF

and its receptor are expressed by a wide range of cells including eosinophils, mast cells, and Th2 cells themselves (156).

We have found site-specific induction of homologs of *Ym1* and *Fizz1* (*AMCase* and *Fizz2*) during infection with *N. brasiliensis*. The consistent expression of this family of proteins, named *ChaFFs* (chitinase and *Fizz* family members) during helminth infection, as reported by our group and others (157), suggests an important regulatory and/or effector function that must be further investigated. We have recently found *Ym1* and *Fizz1* expression in LN APCs during filarial nematode infection, suggesting that they may influence immune responses to filarial parasites. A consistent property of AAMΦs from several different studies is the capacity to induce Th2-cell differentiation (137, 143, 158, 159). Thus, one important function of these cells may be to maintain a potent Th2 environment, presumably detrimental to the parasite.

Of all the potential functions of AAMΦs, one that seems most consistent with the overall expression profile is that of mediators of wound repair. The role of arginase 1 in tissue remodeling is well established, as L-arginine metabolites are consumed in cell proliferation (polyamines) and collagen production (proline), respectively (138, 160). Similarly, *Fizz1* has proliferative and angiogenic properties, stimulating actin and collagen expression (145, 161). Finally, through its carbohydrate-binding properties, *Ym1* could contribute to extracellular matrix deposition during tissue remodeling. Homologs of *Ym1* in *Drosophila* and humans have additional mitogenic properties (162, 163). Thus, the most abundant proteins produced by these macrophages (*Ym1*, *Fizz*, and arginase) are all directly or indirectly implicated in tissue repair. Additionally, we have evidence that AAMΦs produce TGF-β (159) as well as large quantities of IL-6 (Nair and Allen, unpublished observations), two cytokines with strong fibrogenic properties (164, 165).

As our work on macrophages recruited to a broad variety of helminth infections is examined, a common theme has begun to emerge that is directly consistent with the work of Wynn and colleagues (166). One of the most important functions *in vivo* of AAMΦs is to mediate tissue repair. That helminths induce a rapid wound healing response may not be so surprising, as physical trauma to host tissue is a direct result of infection, not only during migratory stages but also at the final site of residence. Adult filarial parasites are highly motile, and the ‘filarial dance’ *in situ* (167) can cause local lymphatic trauma and physical damage (168). Within the gut, blood-feeding nematodes cause repeated intestinal wall injury in the process of feeding (169). When all proceeds well, a fibrogenic response leads to appropriate tissue repair, such as that seen following larval migration through the lung by *N. brasiliensis* or

Ascaris lumbricoides (170). However, when the insult cannot be resolved, fatal fibrosis, such as schistosome-induced periportal hypertension, may develop (171). The balance between these outcomes is highly regulated to minimize end-stage damage by means of the IL-13 decoy receptor to absorb fibrogenic-promoting IL-13 (105), as well as by expression of IFN- γ for its anti-fibrogenic properties (165, 172). As discussed earlier, Tregs are also essential to control of this pathology (18). The necessity to regulate the immune response may thus come not only from the parasite's need to block effector function but also the host's requirement to prevent tissue damage and fibrosis.

Systems immunology

Helminth infection, as discussed above, can have a broad impact on the whole immune system, such as in the example of biasing third-party response toward a Th2 outcome. An interesting possibility is that Treg activity, amplified by helminths, may spread suppression to non-cognate antigens (16). In fact, T-cell mitogen responsiveness is compromised in heavily infected onchocerciasis patients, as are responses to third-party antigens (173, 174) and common vaccines (175–178). These findings may be consistent with a regulatory network that is driven by parasite-specific T cells, but is mediated by non-specific down-modulating cytokines. If present in sufficient numbers, suppression may become non-specific in scope, and thereby alter the whole immune status of the host. We discuss this concept here in the context of co-infection and the susceptibility to allergies and autoimmunity.

Impact of helminths on concurrent/secondary infection

The evidence that helminth infection can alter immune responsiveness to a second infection is irrefutable. A more important issue is how this modulation influences the host in terms of reduced or increased immune pathology and parasite intensity. Not surprisingly, the answers to these questions can differ radically depending on which pathogens are studied and the species of co-infected host.

The question of helminth-malaria co-infection is one with very real world consequences. If as has been suggested helminth co-infection ameliorates cerebral malaria (179), then mass anti-helminthics may have disastrous consequences where malaria is endemic. Alternatively, the additional burden of helminth infection may increase the severity of malaria infection (180). Helmbly and colleagues (181) have investigated the impact of schistosomiasis on malaria in a mouse model. C57BL/6 mice doubly infected with *Plasmodium chabaudi*

and *S. mansoni* had significantly higher malaria parasitemias than singly infected mice, and this outcome was accompanied by reduced TNF- α . Our own work (Graham, Read, Lamb & Allen, unpublished) has elucidated an intriguing relationship regarding MF⁺ versus MF⁻ individuals in malaria-filaria co-infection. BALB/c mice were infected with *L. sigmodontis* and then injected with *P. chabaudi* merozoites after filarial patency. In *L. sigmodontis* infection, approximately 50% of BALB/c mice have 'occult' filariasis with fertile adult worms but no circulating microfilariae. We found that co-infection significantly enhanced the severity of malaria but only among MF⁻ mice. For a given malaria parasite burden, MF⁻ mice suffered more severe malaria in terms of weight loss and anemia, and these symptoms were associated with elevated inflammatory responses. The data strongly suggested that the penalty for the host of co-infection did not occur in MF⁺ animals, because they were able to downregulate the more severe consequences of the pro-inflammatory response. As *P. chabaudi* is not a model for cerebral malaria, these studies did not answer the important question of whether helminth infection can protect against this form of lethal pathology, perhaps through suppression of TNF- α as seen in the Helmbly study. However, in the *P. berghei* model, mice exposed to filarial infective larvae do not develop cerebral malaria, significantly prolonging their survival (182).

The consequence of a pre-existing helminth infection on leishmaniasis has been the subject of several investigations because of the power of the *L. major* murine model to address fundamental immunological questions. Two studies of *S. mansoni* infection in mice have demonstrated that an established helminth infection can delay lesion development (183, 184), while a study with *N. brasiliensis* showed no effect on disease outcome, despite a systemic Th2 bias due to nematode-infected mice (185). Our own work using *L. sigmodontis* and *L. major* is strongly in keeping with the schistosome studies; another striking example of how these broadly divergent parasites can have remarkably similar impacts on the host. We found that the immune responses to *L. major* and *L. sigmodontis* were highly compartmentalized and appropriately polarized, with a type 1 response to *L. major* in the popliteal LNs and a type 2 response to *L. sigmodontis* in the thoracic LNs (Lamb, Graham, Le Goff, Read & Allen, unpublished). However, despite a clear delineation of the immune response in co-infected animals, the impact of helminth infection was still sufficient to delay footpad lesion progression.

A common theme in many helminth co-infection studies is a downregulated pro-inflammatory response. This may have two very different consequences for the host: a beneficial reduction in immunopathology or, conversely, disease exacerbation due

to inadequate control of parasite replication. One of the many challenges to co-infection research is to distinguish the effects of co-infection on parasite control from immunopathology. The infectious disease community is showing an increasing willingness to tackle these complexities experimentally, both in the laboratory and in the field. Certainly, the importance of co-infection dynamics to public health policy cannot be ignored. Not surprisingly, studies to date have generated a wide range of diverse outcomes but also many remarkable similarities. As more studies are performed, generalities regarding the ability of helminth infection to influence the outcome of particular classes of pathogen may emerge. We look forward to further understanding how particular cytokine and cellular networks determine the nature of the interaction between the immune responses to two (or more) distinct pathogens.

Helminths and allergy

The remorseless rise in allergic diseases in the Western world is exciting great interest into the possibility that a corresponding decline in infectious diseases may be responsible. Various forms of this ‘Hygiene Hypothesis’ have been developed since it was first formulated by Strachan (186). In its earlier forms, the hygiene hypothesis postulated that in the absence of childhood bacterial infections, the immune system received insufficient Th1 stimulation, and consequently, Th2-mediated pathologies such as asthma became more prevalent. This challenging proposition did not, however, account for two key observations: that Th1-mediated autoimmune diseases (such as diabetes) are rising in concert with Th2-type allergies, and that in tropical countries with high levels of Th2-driving helminth infections, allergies are less common (187).

A conceptual breakthrough in this area was made by Yazdanbakhsh and colleagues (188) in a study of human schistosomiasis. These workers showed that fewer schistosome-infected children were allergen-reactive in skin tests than uninfected classmates (188), confirming that a Th2-inducing parasite can counteract a Th2-associated pathology. Significantly, schistosome patients who retained their atopic reactivity were shown to produce very little IL-10 in response to schistosome antigen challenge, while infected subjects who were allergy free made high levels of this cytokine. These findings laid the basis for a new regulatory hypothesis, in which a helminth-induced regulatory network dampens responses to allergens (16, 187, 189, 190).

We have tested this hypothesis in experimental mouse models of airway allergy. We first tested mice infected with

the nematode *H. polygyrus*, a parasite which is entirely gastrointestinal in habitat, and sensitized either BALB/c mice with ovalbumin or C57BL/6 mice with house dust mite allergen Derp1. In both models, infection results in substantial depression of airway allergy (measured by eosinophil infiltration into the bronchioalveolar fluid or by epithelial goblet cell proliferation). Parallel results have been obtained in *L. sigmodontis*-infected animals (Wilson, Taylor, and Maizels, unpublished results); thus, suppression of allergy has been observed in two nematode infections, of two different strains of mice, and with two allergens.

We then showed in the *H. polygyrus* model that suppression of airway allergy in infected animals is not accompanied by any switch from Th2 toward Th1 reactivity in LN cells recovered from mice. The protective effect of infection, however, can be reduced by treatment with a depleting antibody to CD25, implicating the involvement of Treg cells in allergic suppression. This possibility was pursued by adoptive transfer experiments, in which mesenteric LN cells (MLNCs) were transferred from an infected, allergen-naïve animal to an uninfected, allergen-sensitized mouse. CD4⁺ CD25⁺ T cells alone could transfer suppression of allergy, although significant effects could also be observed with other populations (Wilson, Taylor, Balic, Lamb & Maizels, unpublished).

The suppression of allergy by transfer of T cells from a nematode-infected animal allowed us to test the hypothesis that polyclonal IgE production is responsible for the lowering of allergy in helminth infection (191). It is known that most helminths stimulate a substantial rise in non-parasite-specific IgE in infection, and it is possible that this competes with allergen-specific IgE for FcεRI receptors on the mast cells which play a crucial role in patent allergy. We measured levels of both allergen-specific and polyclonal IgE in mice displaying suppressed allergy following receipt of MLNCs from *H. polygyrus*-infected donors, and these were comparable to those of control animals. Thus, in the transfer model at least, reduction of allergy was not due to saturation of mast cell FcεRs with allergen-unreactive IgE antibodies.

A further important question was the role of IL-10, which is involved in many facets of allergy downregulation (192). We have found that MLNCs from *H. polygyrus*-infected animals mount a strong antigen-specific IL-10 response on *in vitro* challenge with parasite antigens, and these cells have other characteristics of Tregs (Wilson, Finney, and Maizels, unpublished observations). However, neutralization of IL-10 action through anti-IL-10R antibody administration *in vivo* did not reverse suppression of allergy, and in fact, we have been able to adoptively transfer suppression with MLNCs from an

IL-10^{o/o} mouse into a naïve recipient (Wilson and Maizels, unpublished observations). Perhaps, as in *L. sigmodontis* infections, IL-10 does not play a directly suppressive role in modulating highly polarized Th2 responses.

A summary of our findings in this system is shown in Fig. 9. It has been noted above that *H. polygyrus* is known to down-regulate host immune responses, and the same parasite has been reported to alleviate food allergy (193) and chronic gut inflammation (194). Our work is the first, however, to show systemic effects beyond the gastrointestinal setting and to show that downmodulation is transferable with an identifiable cell population. Moreover, the observation that infection suppresses allergy is not restricted to *H. polygyrus* and *L. sigmodontis*. Similar findings have been reported with other nematode parasites, *Strongyloides stercoralis* (195) and *N. brasiliensis* (196), although the mechanisms responsible have yet to be identified. Significantly, bacterial infections can also exert the same effect, and in one system at least, suppression has also been shown to be mediated by Treg populations (197).

The decline in parasite infections, and in the resultant level of immune downregulation, offers a plausible explanation for the rapid rise in allergies in the developed world. However, even now only a minority of infection-free residents of the West suffer from asthma and other allergies, leading to intense searches for polymorphisms which may render humans more or less susceptible to these diseases. For example, allelic differences in Th2-associated genes, such as IL-13 and signal transducer and activator of transcription 6 (STAT6) (198), show significant association with asthma. A fascinating discovery has now emerged: the same STAT6 polymorphism associated with susceptibility to asthma is, in China, linked to resistance to *A. lumbricoides* infection (199). Thus, predisposition to allergy

may be an evolutionary consequence of heightened immune responsiveness to helminth parasites.

Molecular basis of helminth immunomodulation

How helminths downmodulate host immunity at the molecular level is the subject of intense research at the reductionist, gene-by-gene level (200). Genomic and expression-based analysis of parasitic helminths of veterinary and medical importance as well as model systems has yielded a fascinating crop of potential immunomodulators. Viral pathogens devote a considerable portion of their compact genomes to immune evasion products with fascinating properties (201). In comparison, it seems likely that helminth genomes (1–3 × 10⁸ bp, approximately 20 000 protein-coding genes) will be large-scale repositories of novel mediators, with exciting potential both for advancing our understanding of parasitism and for our capacity to regulate immune responses in pathology (202).

The helminth immunomodulators so far discovered are generally homologs of mammalian immune system genes. Unlike certain viruses, which have captured host cytokine genes, no evidence for horizontal gene transfer from mammals to helminths has yet been found. Most of the gene products described below are members of ancient gene families that have evolved in parallel in the vertebrate and invertebrate lineages; interestingly, if parasitism has been a relatively recent adaptation in evolutionary time, we predict that there must have been convergent evolution in which parasite genes optimize their effects on mammalian immune receptors.

In the following sections, we discuss some major molecular entities from the nematode parasites we are currently studying; we focus on immunological properties, as the molecular

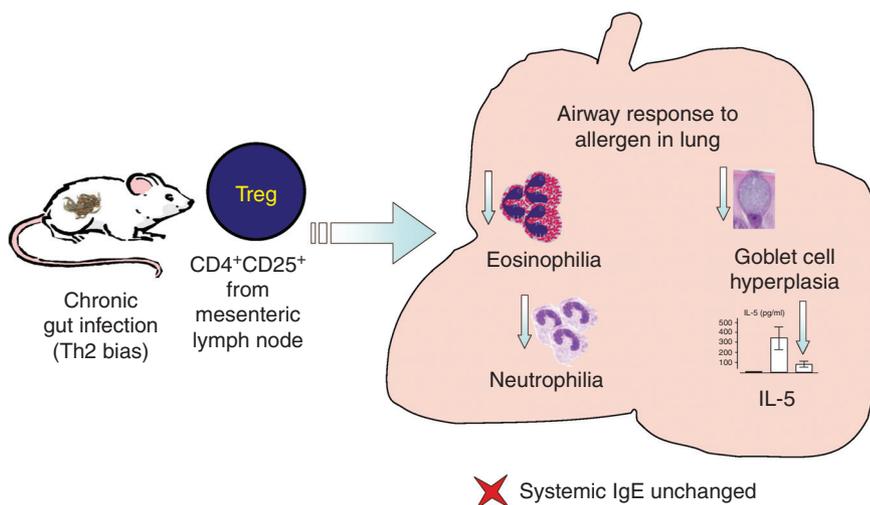


Fig. 9. Chronic helminth infections can suppress allergic reactions, even where there is no physical intersection between parasite (gut) and allergy (lung).

features of these genes has been recently reviewed in more detail (200, 202). We then discuss which, if any, of these stimulate the host immunoregulatory network, and if so, whether the host is reacting to specific molecular patterns of helminths or whether parasites have evolved to drive regulation for their own ends.

Genes and genomes

Helminth biology is on the verge of a transformation, as the first complete genome sequences near publication (203). Genomics will soon provide a series of telling insights into the evolution of parasites, identifying large numbers of predicted proteins from helminths, constructing a detailed picture of conserved biochemical pathways, and identifying potential immunological mediators. How will such an enormous richness of data – perhaps 20 000 predicted proteins per parasite – be analyzed and harnessed?

Fig. 10 presents one approach to the analysis of helminth genes, particularly apt for the nematodes. We can consider three overlapping gene sets, from the mammalian host, nematode parasites, and the free-living nematode *Caenorhabditis elegans*. Many immunologically important parasite products are members of widely conserved gene families and are found at the intersection of all three gene sets (Core Animal Genes). These will include, as discussed below, cytokine homologs and protease inhibitors. A second category will be nematode specific, either having arisen within the nematode lineage or having diverged too far from the vertebrate comparator for any sequence similarity to be discerned. This group (Common Nematode Genes) will include housekeeping proteins necessary for nematode physiology but is likely to also

encompass products that contribute to parasitism. Finally, the third category (Novel Genes) will be genes that are entirely novel and not present in *C. elegans*; we expect many of the key proteins required for parasite success to be within this section.

Estimates of how many genes are in each category will vary with the datasets in question and the threshold for deeming similarity. A recent example, based on *B. malayi* expressed sequence tags (ESTs), took 6822 partial gene sequences (clusters) and reported that, at a relatively stringent threshold, 1748 were homologous to non-nematode sequences (25.6% Core Genes); 793 were similar only to other nematode sequences (11.6% Common Nematode), and the remaining 4281 had no database homolog (62.7% Novel Genes) (204). In a smaller study on *N. brasiliensis*, ESTs were distributed as 35.9% Core Genes, 27.4% Common Nematode, and 36.6% Novel Genes (205). The higher proportion of *N. brasiliensis* genes with homologs in *C. elegans* reflects a close phylogenetic relationship between these two species, but as in *Brugia*, a substantial number of new genes have been discovered with no similarity to existing database sequences.

Although the scale of whole-genome analyses of helminths is very impressive, immunologists may need to focus on a more restricted set of proteins to provide a manageable number of candidates for experimental work. In particular, secreted proteins would be a rational subset to analyze, as these proteins are likely to represent the principal immunologically active products, if only on the premise that a protein must be exported if it is to exert an effect on the host system.

The conventional approach to secreted protein analysis is proteomics, which is being successfully applied to many helminth parasites (206). We have, for example, identified proteins in NES and other nematode secretions by mass spectrometric analysis coupled with database interrogation (Harcus, Maizels, Curwen, Ashton, and Wilson, unpublished observations). In addition, we have tested a genomic pathway to secreted protein analysis, taking advantage of the fact that nearly all secreted proteins contain an identifiable hydrophobic signal sequence at the N-terminus of the newly synthesized protein. We applied this to a study of *N. brasiliensis* ESTs, derived from cDNA libraries, that included a new technique to clone from the 5'-cap of full-length mRNA, thus ensuring the inclusion of the N-terminal sequence (207). Bioinformatic comparison of proteins bearing signal sequences compared to those which did not produced a striking result (205): far more secreted proteins were novel, i.e. had diverged sufficiently rapidly from *C. elegans* (the closest relative in the database) to have lost all recognizable sequence similarity. From this finding, we suggested that the rapid evolution of many

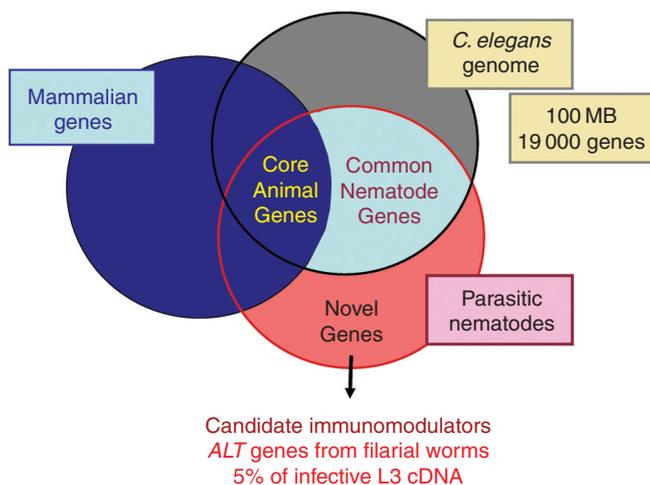


Fig. 10. Hosts and parasites: overlapping gene sets.

secreted proteins may reflect an evolutionary adaption to the demands of parasite evasion of host immunity.

Cytokine homologs

The cytokine network is a central pillar of host defenses against pathogens. It is not surprising therefore to find that one immune evasion strategy developed by infectious organisms is to produce homologs of mammalian cytokines such as TGF- β and macrophage migration inhibitory factor (MIF).

TGF- β is a profoundly downregulatory cytokine that belongs to an ancient gene superfamily, conserved across metazoan organisms, and encompassing many proteins involved in both developmental and immunological processes (208). In *Brugia*, two genes encoding ligands from the TGF- β superfamily have been identified, bearing 28–42% amino acid identity to human proteins. *Bm-tgh-1* is more similar to the bone morphogenetic protein subfamily, which triggers differentiation and growth. This gene is not expressed in microfilaria, an arrested stage of development, and it is present maximally during parasite molting in the mammalian host (209). The second gene *Bm-tgh-2* is more similar at the sequence level to human TGF- β . This gene reaches maximal levels in the microfilaria, but it is also expressed in mature adult male and female parasites. *Bm-TGH-2* has been shown to be secreted by adult worms and to bind to mammalian TGF- β receptors, suggesting that TGH-2 might have an immunomodulatory function in the host (210). As TGF- β has been shown to induce naïve T cells to adopt a regulatory T-cell phenotype (211–213), we hypothesize that parasite-derived TGF- β may drive Treg differentiation, thereby promoting long-term survival of parasites.

Helminths not only express ligands from the TGF- β superfamily but also contain type I TGF- β receptors (214, 215) and downstream signaling components that are functionally interchangeable with mammalian homologs (216). In *Brugia*, *Bp-trk-1* encodes a receptor serine/threonine kinase that shares 67% identity with the kinase domain of SMA-6, a second type I TGF- β receptor from *C. elegans* (217). *Bp-trk-1* is expressed in microfilaria, infective larvae, and adult parasites. The *S. mansoni* type I receptor (*SmRK-1*) (215) shares up to 58% identity with the kinase domain of other type I receptors, but as with *Brugia*, no type II receptor can be identified. Ligand-dependent activation of type I receptors is generally considered to require a type II TGF- β receptor for effective ligand binding. However, in *C. elegans*, the type I receptor DAF-1 can signal autonomously (218), suggesting that *Brugia* TRK-1 and *SmRK-1* may function in a TGF- β signaling pathway without participation of type II receptors alone. Whether or not type II subunits are involved,

the expression of this family of receptors may allow host-to-parasite signaling, a new aspect of the molecular cross-talk in infection.

The macrophage MIF gene family is certainly as ancient as the TGF- β superfamily, and possibly more so, as structural homologs can be found in bacterial organisms. MIF was first discovered as a stimulatory cytokine for macrophages, in *in vitro* studies of delayed-type hypersensitivity (219, 220), but is now recognized as a multipotent activator of the immune system with a generally inflammatory character (221, 222). For example, MIF^{0/0} mice survive the normally lethal administration of LPS or staphylococcal enterotoxin, and they are resistant to trinitrobenzene sulfonic acid-induced colitis (223). MIF is thus involved in both acute septic shock and more sustained inflammatory disease. MIF may also be essential in many infections: MIF^{0/0} mice die following low-dose *Salmonella typhimurium* infection (224) and are more susceptible to *L. major* (225) and *T. crassiceps* (226) parasites. In view of this pro-inflammatory pedigree, it was surprising to discover that MIF homologs are expressed by a number of long-lived nematode parasites that are more associated with anti-inflammatory conditions (227–229).

We decided to look in more detail at the structure and function of the two MIF homologs from *B. malayi*, *Bm-MIF-1* (227) and *Bm-MIF-2* (229). Our first hypothesis was that the parasite proteins would antagonize the mammalian ones or even stimulate host cells in a different way altogether. Surprisingly, tests with human monocytes showed that like human MIF, parasite MIF proteins induce a pro-inflammatory profile of cytokines, such as TNF- α , IL-8, and even human MIF (229). Thus, parasite MIF induces host cells to release more MIF of endogenous origin. Biochemical studies showed that *B. malayi* MIFs also reproduced one of the more enigmatic properties of human MIF, a dopachrome tautomerase enzyme activity. Moreover, as in the mammalian molecule, enzyme function was abolished in the site-directed mutants MIF-1G and MIF-2G, in which a key proline-2 was mutated to glycine (229). Finally, *Bm-MIF-2* was successfully crystallized and shown to have close structural similarity to human MIF, even though only 28% of amino acid residues are shared between the two molecules (229). What, then, was the advantage to the parasite of producing a close mimic of host MIF?

Because of the known activity of MIF for macrophages and the novel phenotype of alternative activation in macrophages from *Brugia*-infected mice, we injected *Bm-MIF-1* (free of detectable LPS) nine times over 3 weeks into the peritoneal cavity (230). The infiltrating population of cells was then tested: although the macrophages were not directly suppressive, they expressed

YM1, and a three-fold rise in eosinophils was also observed. Significantly, such changes were not observed with Bm-MIF-1G mutant recombinant protein or with LPS at a dose corresponding to the threshold of detection in the original MIF-1 preparation (230). YM1 upregulation occurred even in IL-4^{o/o} and IL-5^{o/o} mice, suggesting that MIF may be acting directly to induce YM1 production. From this finding, we have suggested that Bm-MIF may be the first stimulus for macrophages to begin alternative differentiation but that other factors are necessary for this process to complete. These findings help to unravel the central paradox of why parasites secrete products that could intensify inflammatory tissue reactions. It may be that as-yet-unidentified structural differences confer a novel function of parasite MIFs, which result in their exerting a counter-inflammatory influence. However, we also consider that a dynamic hypothesis is likely, in which repeated or continuous exposure to parasite MIFs switches the host immune response into a more counter-inflammatory response, as exemplified by alternatively activated macrophages.

Protease inhibitors

Another category of conserved genes that interacts significantly with the host immune system is protease inhibitors, which can be readily identified by sequence similarities to host genes. The filarial parasites produce at least three classes that are active against aspartyl, cysteine, and serine proteases. Most other helminths studied elaborate similar groups of protease inhibitors with a broad range of functions.

The cysteine protease inhibitor (CPI) family from filarial nematodes has been intensely studied for potential immunomodulatory properties. The filarial CPIs are homologous to cystatins, which are widely expressed in DCs and other immune system cells. Among the functions of mammalian cystatins is the regulation of antigen processing in the highly protease-dependent MHC class II pathway (231). Detailed analysis of cystatins reveals the existence of two inhibitory sites: (i) common throughout the evolutionary tree, blocks classical cysteine proteases such as papain; (ii) found only in certain mammalian cystatins (such as expressed in DCs), independently inhibits a specialized protease, asparaginyl endopeptidase (AEP), which cleaves proteins at asparagine residues (232). Significantly, AEP is a crucial enzyme in the MHC class II processing pathway (233).

Sequence analysis of nematode cystatins provided some intriguing insights. Many homologs, including those from *C. elegans*, do not appear to encode an AEP-inhibitory site, consistent with the evolutionary position of this clade. However, a subset of filarial cystatins displays a very similar amino

acid motif to the mammalian AEP-inhibiting molecules. We demonstrated functionally that *B. malayi* Bm-CPI-2 inhibits AEP in human B cells and indeed will block antigenic peptide presentation through the MHC class II pathway (234). Site-directed mutagenesis of the AEP-inhibiting motif confirmed that the same motif as found in mammalian proteins is involved in enzyme inhibition. We also showed that *C. elegans* CPI proteins carry no inhibitory activity toward AEP (Murray, Manoury, Watts & Maizels, unpublished). These data suggest that Bm-CPI-2 represents a case of microconvergent evolution – the acquisition of a mammalian-like motif that targets a mammalian enzyme, inserted on an evolutionary background from which the motif is altogether absent.

Independently of our work, studies with CPI homologs from *Onchocerca volvulus* and *A. viteae* have focused on their capacity to induce downregulatory mechanisms in human immune system cells (235). In addition to blocking proliferative responses, CPI proteins elicit an IL-10 response from macrophage populations. Because it is established in both *A. viteae* (236) and *B. malayi* (Gregory & Maizels, unpublished) that the CPI homologs are released by living worms, the induction of IL-10 *in vivo* may be a key step in the maintenance of immune regulation during infection.

A second type of inhibitor widely distributed among parasitic helminths is the serine protease inhibitor (serpin) family (237). Taking *B. malayi* again as the example, the major serpin produced is Bm-SPN-2, the most highly expressed protein in the MF stage. Expression is strictly stage specific, as no other point in the life cycle shows detectable *spn-2* mRNA, and the protein has the intriguing property of inducing skewed Th1 responses in the form of IFN- γ release on challenge of mice primed either with SPN-2 or with entire microfilariae. Thus, SPN-2 reproduces the Th1 bias noted earlier to be associated with the MF stage (56). The inhibitory loop of Bm-SPN-2 has an unusual sequence, and we in collaboration with others found that two neutrophil proteases (cathepsin G and neutrophil elastase) were specifically blocked by SPN-2 (238). However, others have reported that they were unable to demonstrate active inhibition by recombinant SPN-2 (239).

The Abundant Larval Transcript antigens

Filarial nematodes share a common, prominent set of antigens that are produced in abundance by the mosquito-borne stage 3 larvae, implying a role in invasion of (and establishment in) the mammalian host. This gene family was independently discovered in several different filarial species, and only distantly related sequences are known from other parasitic and free-living nematodes. The genes were first identified as the major trans-spliced cDNA from *B. malayi* infective larvae (240). Cloning and

comparison with accumulating EST datasets revealed a multi-gene family with at least two members highly expressed in the L3; these two genes were named *Abundant Larval Transcripts* (*alt*)-1 and *alt*-2. Parallel studies have also identified *alt*-like genes in *O. volvulus* (241, 242), *L. sigmodontis* (243), *D. immitis* (244), and *A. viteae* (245). All these members of the ALT family have a common protein structure that can be divided into a signal peptide, a variable and highly charged domain, and a conserved, cysteine-rich domain. However, a single *alt* gene was identified in the genomes of the free-living nematodes *C. elegans* and *Caenorhabditis briggsae*. Genomic analysis of *B. malayi* shows one homolog that does not contain the charged domain (Gregory, Maizels, and Blaxter, unpublished observation). These homologs lacking the charged region have only been shown to be expressed in adult nematodes.

To understand how the parasite manages to synthesize large amounts of two specialized products in a stage-specific manner, the genomic organization of the *B. malayi alt*-1 and *alt*-2 was defined (246). The genomic information showed that *alt*-2 is a single gene locus providing up to 3.2% of cDNA in the L3 stage. *alt*-1 is present as two near-identical copies organized in an inverted repeat of 7.6 kb in a different locus to *alt*-2.

Recognition of the ALT protein has been associated with protective immunity in experimental models of *B. malayi* (247) and *O. volvulus* (241). In mice vaccinated with irradiated *L. sigmodontis* larvae, IL-5 responses to ALT-1 are inversely correlated with worm recovery following challenge, suggesting ALT-1 is an important target of the protective IL-5 response (Taylor and Allen, unpublished observation). With the finding that Bm-ALT-1 elicits 76% protection in jirds (247), the ALTs

have become front-running new vaccine candidates for filariasis and onchocerciasis.

As members of a novel gene family, the function of the ALTs has been elusive. Transgenesis and targeted gene deletion have yet to be established for parasitic helminths, and thus, it is not possible to investigate the biological role of ALT proteins by conventional reverse genetics. To test whether ALT proteins functionally interact with the host immune system, we adopted a novel system for functional testing *in vivo*, by transfection into *Leishmania* species (255). Expression of ALT genes in *Leishmania mexicana* promastigotes showed surface expression of the transgenic protein, and it was found to confer greater infectivity of macrophages *in vitro* and accelerated disease *in vivo*. We also showed that *alt*-transfected parasites are more resistant to IFN- γ -induced killing by macrophages. In contrast, transgenic expression of CPI-2 from *B. malayi* did not alter the phenotype or infection kinetics of *L. mexicana*. Array analysis of mRNA from macrophages infected with wildtype or transgenic *Leishmania* shows upregulation of GATA-3 and suppressor of cytokine signaling-1 transcripts (Gomez-Escobar, Prieto-La fuente, Blackburn, Aebischer & Maizels, unpublished). Upregulation of these factors is consistent with the strong Th2 bias observed in infection with filarial parasites.

The glyco-network

Much of the host-parasite interaction is governed at the molecular level by carbohydrate determinants (248). Detailed analysis of the glycans present in a wide range of parasites reveals few common structures, other than a tendency for fucosylated side chains and a generally primitive (high mannose) content of

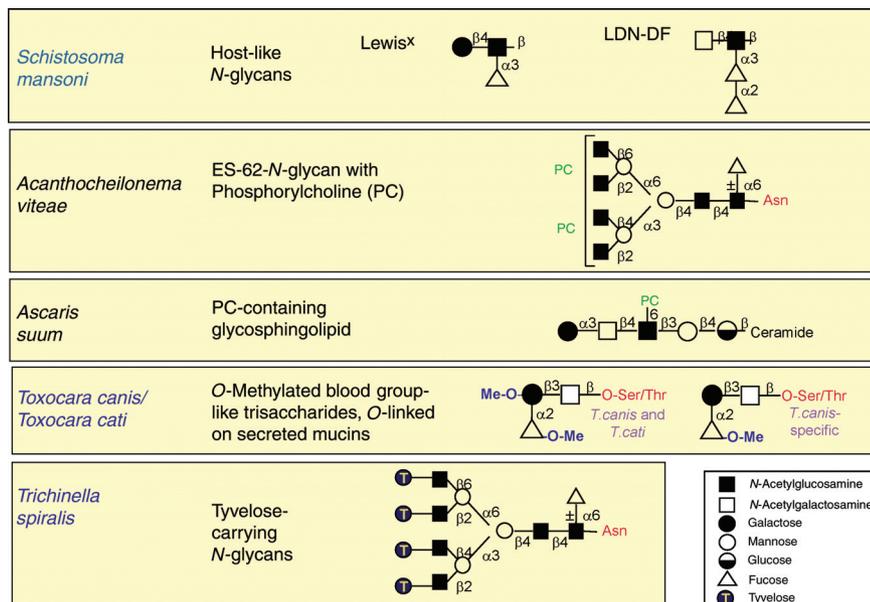


Fig. 11. Helminth glycans.

N-glycans (Fig. 11). No candidate pathogen-associated molecular pattern can be discerned characteristic of helminths as a group or of major subsets such as nematodes or trematodes. Glycans vary from those highly specific for one nematode species, such as the tyvelose-terminating N-linked oligosaccharides of *Trichinella spiralis* (249), to the near-ubiquitous phosphorylcholine-coupled N-linked sugars in different species of filarial nematodes (250). Intriguingly, the trematode *S. mansoni* expresses both unique (251) and host-like Lewis^x-like specificities (252). Similarly, tissue-dwelling stages of *Toxocara* species express a high level of a blood group H-like trisaccharide, which bears novel O-methylation sites (253). Thus, parasite glycans encompass both highly specific and cross-reactive determinants, and it is possible that the latter could operate as molecular mimics of host carbohydrates, perhaps providing false signals or blocking lectin-dependent interactions in the host defense mechanism. While it is generally true that helminth glycans are not substituted with terminal sialic acid (and the absence of this modification could act as a signal to the innate immune system) in at least the instance of *Echinococcus granulosus*, some sialylation is present on parasite glycans (254).

There is a strong case that helminth glycans contribute to Th2 induction, in some of the major species at least. Th2 responses to somatic extracts of adult *Brugia* are diminished upon periodic treatment (5). Synthetic saccharides corresponding to schistosome glycans can initiate a Th2 response (41), and glycoconjugates bearing the Lewis^x determinant activate B1 cells to produce IL-10, favoring a Th2 phenotype (255, 256). The *S. mansoni* egg glycans, specifically Lewis^x, are recognized by DCs through surface C-type lectin receptors, such as DC-SIGN (257). Intriguingly, some parasites themselves make prominent use of lectins, such as the C-type lectins secreted by *T. canis*

(258–260). The lectins and glycans may be two sides of the same coin, a molecular strategy by parasites to interrupt or misdirect host inflammatory responses, such as the selectin-dependent extravasation of leukocytes into infected tissue.

The debate will continue as to whether the host immune system has evolved lectin receptors on DCs as molecular pattern recognition devices to detect helminths or whether helminths have evolved specific ligands to trigger these host receptors. Germane to this argument is the finding that *C. elegans* induces Th2 responses in mice, but less potently than do parasites (5). Does this finding signify that free-living ancestors possessed Th2-inducing ligands which have evolved in parasites to act more effectively?

Conclusion

Helminth parasites are clearly masters in the art of immunoregulation. By studying their ability to manipulate the immune system, we will learn not only how to intervene and cure infections but also perhaps how to imitate helminths in regulating untoward responses in the body. Like so much else in immunology, there are some important complexities that require thoughtful investigation. For example, if we target regulatory mechanisms and unleash the full force of the immune system on resident parasites, will we simply amplify pathology and trigger autoimmunity? Is the relative inefficacy of helminth vaccines due to concomitant stimulation of effector and regulatory populations to parasite antigens? How can we focus intervention at the antigen-specific level, either in the context of disabling parasite-specific Tregs or in the context of exploiting helminth-derived immunomodulators, to selectively turn off effector responses to allergens or autoantigens? We are now turning our attention to these questions.

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